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Research Article

Molecular and in Silico Analysis of *MEFV* Variants in Familial Mediterranean Fever Patients in Southwest Iran

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Abstract

Familial Mediterranean Fever (FMF) is classified as an autoinflammatory genetic disease inherited by mutations in *MEFV*. These mutations can affect the dysregulation of inflammatory processes in the human body and lead to fever and pain in the chest and abdomen. Many known missense mutations in *MEFV* are linked to FMF disease. Mutations in

MEFV in most cases are located on the short arm of chromosome 16 and can impair the function of the pyrin protein. In this research, we aimed to examine the entire exons of *MEFV* for 13 cases (8 females and 5 males) with FMF diagnosis from Southwest Iran. Hence, we amplified and sequenced the exons of *MEFV* and then, in-silico analysis of detected changes was applied to estimate the probability of pathogenicity for the identified variants. Finally, we found five single nucleotide substitutions, including M694V (c.2080A>G), R202Q (c.605G>A), E447G (c.1430A>G), E148Q (c.442G>C), and V726A (c.2177T>C), in the under-represented patients. The most frequent mutations in our study were R202Q (38.46%) within exon 2 and M694V (30.7%) within exon 10. Other mutations accounted for a further 23% of the alleles, including E477G (7.6%), E148Q (7.6%), and V726A (7.6%). According to the *in-silico* analyses, including variation pathogenicity, protein structure, and allele frequency assessments, we concluded that all these variants could be considered in FMF molecular profiling in southwest Iran.

Keywords
Autoinflammatory disease; familial Mediterranean fever; *MEFV* mutations; missense mutations

1. Introduction

Familial Mediterranean Fever (FMF, OMIM# 249100) is a hereditary autoinflammatory disease caused by gain-of-function mutations in *MEFV* [1]. This gene is located on the 16p13.3 chromosome, comprised of 10 exons with 21600 base pairs which can make a 781-amino acid long protein called pyrin [2, 3]. Pyrin is essential in the immune system and regulates inflammation processes by attaching to additional proteins forming the inflammasome complex [4, 5]. The *MEFV* mutations directly affect pyrin protein activity by disrupting the balance between different inflammation factors, which may cause pain and fever in the chest, abdomen, and joints [6]. To date, mutations reported from different populations for FMF include Jewish, Armenian, Turkish, and Arab people with different frequencies [7]. From reported changes in the *MEFV*, five missense mutations namely E148Q, M694I, M694V, M680I, and V726A count as the most common reported mutations [8, 9]. The major site of mutations in exons 10 and 2 has a smaller cluster, while exons 3, 5, and 9 have less frequent variants. Since this disease has a wide range of signs and symptoms, it is necessary to use molecular techniques to help with clinical diagnosis. Hence, in this study, we determined pathogenic variants of *MEFV*, coupled with bioinformatics analysis, in FMF patients with clinical manifestations of persistent fever and abdominal pain referring to Narges Genetic Laboratory. This study can help with a final diagnosis of *MEFV* mutation distributions in Iranian patients from southwest of Iran.

2. Materials and Methods

2.1 Editorial Policies and Ethical Considerations

This research study was approved by the Ahvaz Jundishapur University of Medical Sciences Institutional Review. In addition, a declaration of consent was obtained from participants.

2.2 Study Design and Patients

For the current study, the confirmation of FMF diagnosis for the patients referred to Narges Genetic Laboratory in the southwest of Iran using molecular techniques was performed [10]. The inclusive enrolment dates of this study were from July 2013 to June 2022. Moreover, the individual signed information according to the ethical requirements.

2.3 Peripheral Blood Samples

Blood samples (10 ml) were obtained from all enrolled FMF patients and their parents in EDTA tubes.

2.4 Genomic DNA Extraction

Genomic DNA was extracted from the 10 ml peripheral blood samples using the salting-out technique obtained from subjects [11]. Next, the quality and the quantity of the isolated DNA samples were analyzed by gel electrophoresis and Nano-drop (ThermoFisher, Europe).

2.5 Polymerase Chain Reaction

Primer3 tool was utilized to design primers for all exons of *MEFV* [12]. For PCR, Master Mix (Ampliqon Denmark) was used. The first step was performed at 93°C for 5 min as initial denaturation, then 35 cycles of 93°C for 1 min, annealing and extension steps at 60°C for 30 sec and 72°C for 30 sec respectively. Post-PCR final incubation step of 72°C for 3 min as a final extension. The details of primers are provided in Table 1.

Table 1 Primer sequences used for PCR and Sanger sequencing.

Gene Name	Strand	primers 5'–3'	Product size
*MEFV*_E1	Forward	CAACCTGCCTTTTCTTGCTC	406
	Reverse	CACTCAGCACTGGATGAGGA	
*MEFV*_E2	Forward 1	AAACGTGGGACAGCTTCATC	498
	Reverse 1	CAAGGCTTCTAGGTCGCATC	
	Forward 2	GAGCAAACGCAGAGAGAAGG	576
	Reverse 2	GCTGGTCTCAAAGTCTTGGC	
*MEFV*_E3	Forward	AACTTGGGTTTGCCATTCAG	467
	Reverse	GGGAAAATGAAGTAAGGCCC	
*MEFV*_E4	Forward	TAAAGATGGCAGGAGCCAAA	209
	Reverse	TGCTCACTCTTCCCACCTTC	
*MEFV*_E5	Forward	GTCCACCCACAGCACAGAC	334
	Reverse	AGGCATCCTGATAGGCACAG	
*MEFV*_E6	Forward	AGGAGCCCAGAAGTAGAGGC	195
	Reverse	GAACATCTCCCTCCCAGGTC	
*MEFV*_E7	Forward	TGTGCCCTGTGGAGAATGTA	252
	Reverse	AGTGAGCATGCACCTCCAAT	
*MEFV*_E8	Forward	CTCTCCTCCATCTGTCCCTG	175

	Reverse	CCCTCAAGTCAACAGCACAA	
*MEFV*_E9	Forward	GCCCCTCTTAATGCTAATGC	185
	Reverse	CAGGAAACAGGGACAGGGTA	
	Forward 1	AGGTGGGGAGAACCCTGTAG	497
*MEFV*_E10	Reverse 1	AGATGTGGGATCTGGCTGTC	
	Forward 2	AATAAAGGAGCCTCCCAAGC	473
	Reverse 2	GGATTATGCAACGACTCCGT	

2.6 Gene Sequencing

Mutations in *MEFV* were examined in DNA amplicons using Applied Biosystems 3700 DNA analyzer (sequencer). Results of the Sanger sequencing were evaluated using Chromas LITE2.1.1, and the BLASTN software.

2.7 In-Silico Analysis of Variants

There are many tools for analyzing the pathogenicity potent and structure of variants. In the present study, we used some of these tools for the evaluation of disease-causing potentiality of the variants. In the first step, the reference coding DNA and protein sequence of human *MEFV* wild-type were collected from the Ensembl genome (gene bank transcript ID ENST00000219596, NM_000243) database (https://asia.ensembl.org/index.html). We implemented the program Predict SNP which significantly enhanced prediction performance and simultaneously returned results for all mutations [13]. The Mutation Taster2 can estimate the functional consequence of amino acid residue substitutions, intronic alterations, indel mutations, and variants spanning introns-exon borders [14]. Moreover, SNPs & GO are suitable for predicting whether a specific variation can be categorized as disease-related or neutral. This tool uses the protein function to predict the pathogenicity of missense mutations [15]. Meta-SNP is another method for detecting synonymous SNVs for disease-associated SNVs [16]. SNAP2 is a neural network-based classifier for screening and predicting the functional effects of sequence variants [17]. Mutation Assessor Web server indicates variants to have a low, medium, and high probability of affecting protein function or being natural [18]. SIFT is a computer algorithm that can predict the effect of amino acid substitution on protein function [19].

2.8 Stability Assessment

The predicting protein stability upon mutation of amino acids is evaluated by I-Mutant [20].

2.9 Structural Analysis

Knowing the 3D structure of a protein provides insight into the mechanism of that protein. HOPE is a powerful program that assesses the function and structure of point mutations [21]. This tool predicts the effects of a mutation on protein structure and function by merging all collected data with known properties of the mutated and wild-type amino acids, such as charge, size, and hydrophobicity value [22].

2.10 Secondary Structure Analysis

YASARA is a molecular modeling program that visualizes large proteins [23]. In this regard, mutant and wild-type protein analysis were done with YASARA version 15.6.21. This research study was approved by the Ahvaz Jundishapur University of Medical Sciences Institutional Review Boards. For genetic analyses, informed consent was waived. All experiments were performed in accordance with relevant guidelines and regulations. All patients provided written informed consent to participate in the study.

3. Results

A total of 13 individuals with FMF (8 Females and 5 Males), from the Southwest of Iran with various ethnicities, including Lor, Fars, and Arab, participated in this research (Table 2). Five mutations in *MEFV* were recognized in enrolled patients (Table 3). In Figure 1, we summed up observed genetic variations in the *MEFV* in cases with FMF from southwest Iran.

Table 2 A brief of the description of 13 affected individuals.

Number	Sex	Variant	Zygosity	Exon	Inheritance	
Patient 1	M	M694V	homozygous	10	AR	
Patient 2	F	M694V	homozygous	10	AR	
Patient 3	F	R202Q	homozygous	2	AR	
Patient 4	F	R202Q	homozygous	2	AR	
Patient 5	F	R202Q	heterozygous	2	AR	Father and mother carrier
Patient 6	M	R202Q	homozygous	2	AR	
Patients 7 and 8	F F	M694V M694V	homozygous	10	AR	Father and mother carrier
Patient 9	M	R202Q	homozygous	2	-	Mother carrier
Patients 10 and 11	F M	E477G E477G	homozygous	5	-	Father affected
Patient 12	M	E148Q	homozygous	2	-	
Patient 13	F	V726A	homozygous	10	AR	

Table 3 All detected variations in the enrolled FMF in the following research.

Gene	Gene location	Coding sequence location	cDNA Location	AA change	Mutation Type	Exon number	References
MEFV	g.13221A>G	c.2080A>G	cDNA.2120A>G	M694V	Missense	10	rs61752717
MEFV	g.2165G>A	c.605G>A	cDNA.645G>A	R202Q	Missense	2	rs224222
MEFV	g.13318T>C	c.2177T>C	cDNA.2217T>C	V726A	Missense	10	rs28940579

MEFV	g.2002G>C	c.442G>C	cDNA.482G>C	E148Q	Missense	2	rs3743930
MEFV	g.9455A>G	c.1430A>G	cDNA.1470A>G	E477G	Missense	5	-

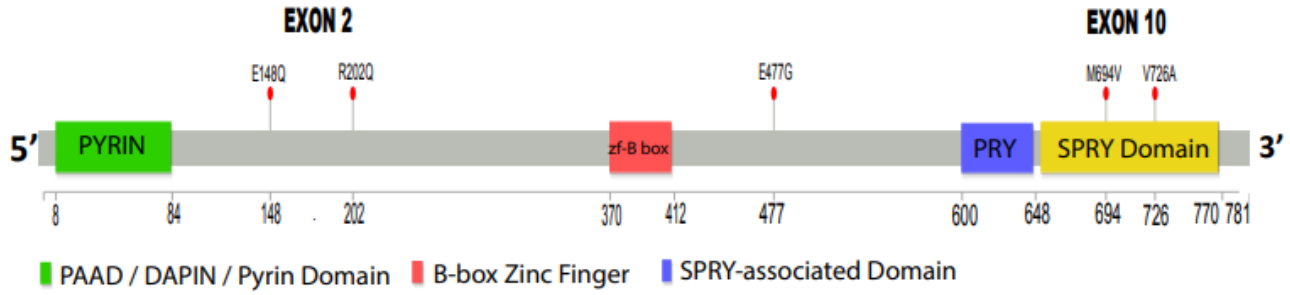

Figure 1 Schematic diagram of the *MEFV* gene depicting the location of the under study variants in *MEFV* Gene.

3.1 Pathogenicity Prediction of Variants

Various Bioinformatics tools were used to predict the disease-causing potentiality of mutations in the *MEFV* of FMF patients. The pathogenic prediction of the variants has been summarized in Table 4. E477G is categorized as disease-causing according to Predict SNP, Meta-SNP, SNP&GO, Mutation Assessor, SIFT and Mutation Taster. According to Predict SNP and Mutation assessor and SIFT E148Q is considered a pathogenic mutation. Mutation Taster V726A was shown as a disease-causing mutation; however, Predict SNP, Meta-SNP, SNP&GO, Mutation Assessor, SIFT and SNAP2 considered V726A as a neutral mutation. Regarding M694V and R202Q variants, SNAP2 and mutation taster suggested they are pathogenic, while Predict SNP, Meta-SNP, SNP&GO and Mutation Assessor showed that they are neural.

Table 4 The results of the pathogenicity prediction of point mutation of *MEFV* gene.

Gene	Variation	Predict SNP	Meta-SNP	SNP&GO (RI)	Mutation Assessor (FI score)	SIFT	SNAP2 (Score)	EXAC (allele frequency & number of homozygotes)	Mutation taster
MEFV	M694V	N, 60%	N, 0.343	N, 6	Neutral, 0.635	Neutral 0.210	Effect, 14	-	P
MEFV	R202Q	N, 83%	N, 0.115	N, 9	Neutral, -0.6	-	Effect, 9	-	P
MEFV	V726A	N, 83%	N, 0.214	N, 8	Neutral, -2.215	Neutral 1.000	Neural, -33	-	D
MEFV	E148Q	D, 51%	N, 0.214	N, 7	Low, 1.83	Disease 0.000	Neural, -23	3.19e-5 Hom = 0	P
MEFV	E477G	D, 76%	D, 0.691	D, 1	Medium, 3.205	Disease 0.000	Effect, 29	-	D

D. Deleterious, N. Neutral, P. Polymorphism, RI. Reliability Index (between 0 and 10).

3.2 Stability Assessment

The protein stability of all variants in the conditions 25°C and pH 7 was assessed, and the data showed that stability of proteins significantly decrease for M694V, E477G, R202Q, E148Q, and V726A variants [Table 5].

Table 5 The result of protein stability of variants by I-Mutant.

Gene	cDNA Location	Variation	pH	Temp. (°C)	Stability (RI)	DDG (Kcal/mol)
MEFV	cDNA.2217T>C	V726A	7.0	25	Decrease, 10	-2.48
MEFV	cDNA.2120A>G	M694V	7.0	25	Decrease, 9	-1.16
MEFV	cDNA.1470A>G	E477G	7.0	25	Decrease, 6	-1.14
MEFV	cDNA.645G>A	R202Q	7.0	25	Decrease, 7	-0.75
MEFV	cDNA.482G>C	E148Q	7.0	25	Decrease, 2	-0.50

RI: Reliability Index/T: Temperature in Celsius degrees/pH: -log[H+].
(DDG: in Kcal/mol DDG < 0: Decrease Stability DDG > 0: Increase Stability).

3.3 Structural Analysis

Based on the Hope results [Figure 2], we provided a summary for evaluating the structural characteristics of variants: M694V can disturb the protein domain and abolish its function because of the different amino acid properties. In comparison to wild-type residue the mutant residue is smaller in size. This mutation is located in a domain important for the binding of other molecules and can cause a possible loss of external interaction [22]. R202Q changes the positive charge of the wild-type into the neutral charge in mutant residue. In addition, the mutant residue is smaller than the wild-type residue, which can cause loss of interactions with other molecules and differences in size. V726A variant in mutant and wild-type amino acids differs in size (mutant residue is smaller). Moreover, because of the changes in the properties of amino acids, this mutation can distribute the protein domain and abolish its function. E148Q variant can change the negative charge of the wild-type residue to a neutral charge in the mutant residue. This alteration causes charge difference and leads to loss of interaction with other molecules or residues. E477G can change the negative charge of the wild-type variant into the neutral charge of mutant residue, which is more hydrophobic than wild-type amino acid. Moreover, since the mutation is located within a stretch of residues (special region), it can affect amino acid properties (Glycin is very flexible), and consequently can disturb the region and function of mutant protein [22].

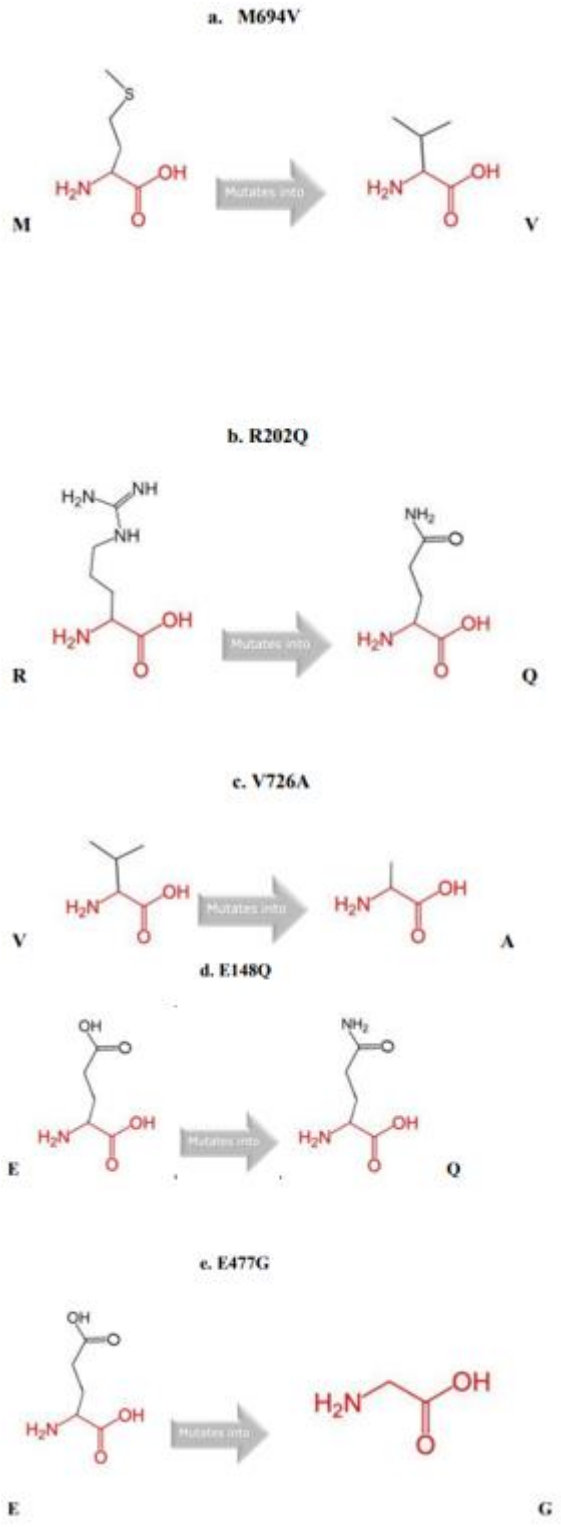

Figure 2 shows the main (left) schematic structures and the altered (right) amino acid. The backbone is colored red (similar for each amino acid residue), while the side chain is colored black (unique for each amino acid residue).

4. Discussion

FMF is an autoinflammatory disorder with worldwide affected individuals (about 120,000 people), and with the highest frequency among ethnic communities of the eastern Mediterranean,

such as Turks, Jews, Armenian, and Arabs (with the highest number in Turkey) [24]. Mutations in the *MFEV* gene cause this autosomal recessive disorder [25, 26]. From the molecular genetic view, it is vital to predicting precisely the connection between the pathogenicity of the changes on the DNA level and clinical consequences of observed mutations; for this purpose, computational tools are employed.

In silico approaches are less time-consuming, cost-effective for identifying candidate mutations, and suitable for genetic studies [3]. Over 333 mutations have been found in *MEFV* related to FMF disease, mainly in exons 2 and 10 [Figure 1] of *MEFV* with different frequencies among various ethnic groups and people [26-28]. In regional studies, the most common variants are R202Q, M694V, E148Q, M680I, V726A and M694I [27, 29]. The E148Q and M694V are the most changes, especially in Turkey, Israel, North African Jews, and Armenians [30-32]. In the studies of Celep et al. in 2019, the R202Q variant was the most frequent mutation in Northern Anatolia [33]. In Iran, a total of 38 various mutations in the *MEFV* gene have been recognized, and M694V, E148Q, V726A, M680I, and M694I were the most common among Iranian FMF patients. Moreover, in the southwest of Iran, M694V, V726A, and M694I are the most common types of mutations [34]. In the present study, entire exons of the *MEFV* gene were sequenced for 13 patients suffering from FMF in southwest of Iran. All of the patients in our study were diagnosed with FMF with recurrent episodes of fever associated with abdominal pain. The most prevalent mutations in our study were R202Q, followed by M694V. The use of bioinformatic tools performed the prediction of the potential pathogenicity of these variations. To examine the stability of proteins we used I-Mutant3 which showed that the stability of proteins significantly decreases in V726A, M694V, and E477G variants. For structural analysis of proteins of mutations, we used the HOPE® tool. Each amino acid indeed has specific biochemical and biophysical characteristics and amino acid change can alter the structure and function of their proteins. Based on Hope analysis all mentioned variants can disturb some intra and inter action of proteins. Although R202Q and E148Q are suggested as polymorphism, changes in the positive charge of the wild-type into the neutral charge in mutant residue in R202Q and negative charge of the wild-type into the neutral charge in mutant residue in E148Q can reduce the interactions with other molecules. Finally, we suggest that all mentioned variants in this study are probably deleterious and can be used to diagnose FMF.

5. Limitation

This study has investigated the molecular analysis of MEFV variants in southwest Iran. Our results were analyzed from bioinformatics to gain a deeper understanding of variants. However, the scope and findings of our study were limited by the number of 13 FMF patients who chose to seek clinical testing and consultation from our qualified healthcare professional.

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Author Contributions

HG and MZ conceptualized the study; NN collected the Data, wrote the original draft, prepared Figures 1 and 2; JZ, FF, and SP were responsible for PCR and Sanger sequencing; H.G, MH, AS, and GS provided supervision; MZ & HG & RG reviewed the original draft.

Competing Interests

The authors declare that they have no competing interests.

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